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DOI:
10.1016/j.psyneuen.2017.09.021

Document Version
Publisher's PDF, also known as Version of record

Link to publication record in King's Research Portal

Citation for published version (APA):
Kumsta, R., Schlotz, W., Golm, D., Moser, D., Kennedy, M., Knights, N., Kreppner, J., Maughan, B., Rutter, M., & Sonuga-Barke, E. (2017). HPA axis dysregulation in adult adoptees twenty years after severe institutional deprivation in childhood. *Psychoneuroendocrinology*, *86*, 196-202. https://doi.org/10.1016/j.psyneuen.2017.09.021

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Download date: 05. May. 2023

Psychoneuroendocrinology 86 (2017) 196–202

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

HPA axis dysregulation in adult adoptees twenty years after severe institutional deprivation in childhood

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ARTICLE INFO

Keywords:
Institutional deprivation
Early trauma
Cortisol awakening response
Diurnal cortisol slope
ERA study

ABSTRACT

Hypothalamic–pituitary–adrenal (HPA) axis function is disrupted in institutionally-deprived children – reduced morning cortisol, flattened diurnal slope and blunted reactivity persist even after successful adoption into positive family environments. Here we test whether such effects persist into adulthood. Cortisol release across the day (sampled at awakening, 30 and 45 min later, and at four points across the day) was investigated in young adult adoptees who had lived in severe deprivation for up to 43 months in early childhood in Ceaușescu's Romanian orphanages and a comparison group of non-deprived UK adoptees (Total N = 57; mean age = 24 ± 0.9 years). The mediating role of cortisol levels on adult mental health was examined using data from standardized clinical assessments. Cortisol profiles were disrupted in the Romanian adoptees who experienced more than 6 months deprivation marked by a striking absence of the cortisol awakening response (CAR) and a significantly flatter cortisol curve until 1 h 15 min after awakening. Whereas institutional deprivation was associated with both cortisol secretion and emergence of emotional problems in young adulthood, path analysis revealed no evidence for a mediating role of CAR disruption in the sub-sample studied here. The results are in line with findings of HPA axis hypo-functionality following early adverse experience and provide strong evidence for long-term programming effects of HPA axis function through experience of institutional deprivation.

1. Introduction

Time-limited exposure to environmental adversity during childhood is associated with negative outcomes in adulthood (Gilbert et al., 2009; Sonuga-Barke et al., 2017). How such early life influences become "biologically embedded" to produce such long-lasting effects remains an unresolved question. Biological programming hypotheses implicating different biological systems have been proposed (Hertzman, 2012). The hypothalamic–pituitary–adrenal (HPA) axis has been a focus of enquiry because of its role in mediating the stress response, which when disrupted can cause mental ill health (Lupien et al., 2009), and evidence from experimental animal studies of a link between early-life stress and long-term HPA dysregulation (Sanchez, 2006). It has been challenging to validate these results in humans given justifiable constraints on exposing participants to adverse experiences. Although the majority of studies in adults reporting adversity in childhood have found HPA axis dysregulations (Carpenter et al., 2007; Elzinga et al., 2008; Lovallo et al., 2011; MacMillan et al., 2009; Power et al., 2012; Schwaiger et al., 2016), linking these effects to early life adversity per se, rather than, for instance, later emerging mental health problems is problematic, given the retrospective, and potentially questionable nature of the reports of the nature and timing of exposures (Rutter et al., 2010).

Prospective cohort studies of children exposed to institutional deprivation and then either fostered or adopted offer a more powerful test of the impact of early adversity on HPA axis function. This is because

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http://dx.doi.org/10.1016/j.psyneuen.2017.09.021
Received 4 July 2017; Received in revised form 26 September 2017; Accepted 26 September 2017
0306-4530/ © 2017 Published by Elsevier Ltd.

they more precisely characterize the nature and timing of adverse exposures and, crucially, allow the possibility of testing for a relationship between deprivation dose and HPA axis functioning – a necessary, though not sufficient condition, for inferring causation in observational studies (Rutter et al., 2010). However, while these studies often support a link between early deprivation and alterations in both unstimulated and stress-induced HPA axis regulation, they have so far included only relatively short follow-up periods (into early and middle childhood). Koss et al. found blunted HPA axis reactivity in deprived and adopted one to three year old infants relative to non-adopted controls during the transition to adoptive family care − effects that persisted for a number of years post adoption (Koss et al., 2014; Koss et al., 2016). The Bucharest Early Intervention Project reported a blunted cortisol response in children who remained in institutional care compared with children randomized to foster care before (rather than after) age two years – providing evidence of a relationship between deprivation dose and HPA effects (McLaughlin et al., 2015). One long-term follow-up into adulthood found lower morning cortisol levels and flatter diurnal slopes in adoptees who experienced severe neglect or abuse compared to non-abused individuals (van der Vegt et al., 2009). It is of note that these effects were only found in those with anxiety disorder – possibly reflecting vulnerability to mood disorders in those with HPA axis dysregulation. Alternatively, altered cortisol regulation might be a consequence of anxiety disorders (van der Vegt et al., 2010).

In the current study we examine, for the first time, whether institutional deprivation early in childhood is associated with HPA axis dysregulation in adulthood in a sample where the specific timing and duration of deprivation is known – thus allowing the impact of dose of deprivation to be established. The English and Romanian Adoptees study is a prospective longitudinal study of the effects of time-limited severe early institutional deprivation in early childhood on development across the life span through to adulthood (Rutter et al., 2010). It utilizes a unique natural experiment whereby children who entered the depriving institutional environments of the Ceausescu regime in the first weeks of life (Rutter et al., 2012) then spent up to 43 months suffering a severe lack of cognitive and social stimulation, impoverished diets and poor hygiene, were subsequently adopted into radically different enriched adoptive home environments in the UK. Importantly, the timing of these moves can be precisely documented for each child (Kumsta et al., 2015). These individuals are compared to a group of non-deprived UK adoptees. All have since been followed up into young adulthood. Findings to date have shown that mental health and neurodevelopmental outcomes are conditional on extended exposure to deprivation (Sonuga-Barke et al., 2017). Those with less than 6 months exposure differ little from the UK controls. However, for those who were in the institutions for more than 6 months there is a complex pattern of persisting neuro-developmental problems—termed deprivation specific patterns, DSP (Kumsta et al., 2010)—marked by quasi-autism (QA); disinhibited social engagement (Kennedy et al., in press); and attention deficit hyperactivity disorder (ADHD Kennedy et al., 2016). Initial cognitive impairment had largely resolved by young adulthood (Sonuga-Barke et al., 2017). Interestingly, problems of anxiety and mood, most commonly associated with HPA axis dysregulation were absent in this group during childhood and early adolescence, but emerged very strongly in adulthood in a way that was consistent with a model of latent vulnerability (McCrory et al., 2017; Sonuga-Barke et al., 2017).

The aims of the current study were therefore to test for i) evidence of a relationship between deprivation and HPA axis dysregulation in terms of cortisol release following awakening and across the day and ii) whether such variations mediated the late onset of emotional problems. We hypothesized that HPA axis functioning would be altered in the Romanian adoptees compared to the non-deprived UK controls – marked by a reduction in cortisol release; that these effects would be most marked in those with extended deprivation; and that these effects would mediate the late onset of emotional problems in the Romanian adoptees. In addition, we investigated the role of DSPs in the association between deprivation, cortisol release and emotional problems.

2. Materials and methods

2.1. Participants

Almost all ERA participants were placed into institution within the first two weeks after birth. They were adopted from Romania into families resident in England between February 1990 and September 1992 and were aged below 43 months of age at the time of UK entry (see Rutter et al., 2010). The ERA study enrolled roughly equal numbers of children adopted before 6 months, between 6 and 24 months and over 24–43 months and compared them with a group of 52 UK-born children adopted before the age of 6 months and not exposed to early deprivation. Development has been assessed at ages 4, 6, 11, 15, and around 23 years (young adulthood). Ethical approval was received from the University of Southampton Research Ethics Committee. All adoptees and family members gave written informed consent.

As in previous analyses we categorized Romanian adoptees into those with 6 months or less (Rom $<$ 6) and those with more than 6 months (up to 43 months; Rom $>$ 6) deprivation experience. A third group comprised UK adoptees (UK; see Table 1). The current investigation was part of the ERA young adult follow-up, and 57 individuals participated in the cortisol arm of the study (see Supplement 1 for reasons for non-inclusion). The study sample did not differ from the original sample in terms of a number of psychosocial and physical characteristics (see Supplemental Table 1). All statistical models were controlled for sex and modelled the data under the missing at random assumption (Schafer and Graham, 2002).

2.2. Design and procedure

Saliva samples were collected on two non-weekend days immediately after awakening (S1), and 30 (S2) and 45 min (S3) thereafter to assess the cortisol awakening response (CAR) (Stalder et al., 2016). Additional samples were collected at 0900 h (S4), 1200 h (S5), 1600 h (S6) and 2000 h (S7) to assess the diurnal cortisol slope (DCS) (Adam and Kumari, 2009). Participants were provided with an instruction booklet and 14 labeled Cortisol Salivettes (Sarstedt, Leicester, UK), informed face-to-face about the sampling protocol, and instructed to adhere to the sampling schedule as closely as possible. To maximize adherence, an individual assessment plan was developed to assist participants with sampling (see Supplement 2). In addition, a reminder text message was sent five minutes before each sample was due.

2.3. Emotional problems and chronic stress

Four measures of emotional problems were used: parent reported anxiety and depression symptoms in young adulthood and change scores of emotional problems between age 15 and young adulthood (self- and parent-report). Anxiety and depression scores were derived from the Conners Comprehensive Behavior Rating Scales (Conners, 2008). The CBRS is an established measure of childhood and adolescent emotional, behavioural and social disturbances, including symptoms of General Anxiety Disorder (GAD) and Major Depressive Episode (DEP). The version used in this study has been adjusted for use in young adulthood. An anxiety score was calculated by averaging all 14 items of the GAD subscale. The same was done for the 15 items of the DEP subscale.

To calculate change scores of emotional problems a selection of items from the Strengths and Difficulties Questionnaire (Goodman, 1997) at age 15 and the CBRS in young adulthood was used (worry, social anxiety and depressed mood) as recently reported (Sonuga-Barke et al., 2017; see also Supplemental information 3). Change scores were calculated by subtracting the age 15 symptom score from the score in

Table 1
Characteristics of study sample (all at young adult follow-up assessment, except where stated differently).

	Group			
	UK	Rom < 6	Rom > 6	Test statistic
n	13	19	25	
Age at assessment (years)[a]	23.6 (0.5)	23.6 (0.9)	24.6 (0.7)	$F_{2,53} = 15.2$, $p < 0.0001$
Sex male	8 (62%)	8 (42%)	6 (24%)	Fisher's exact, $p = 0.073$
Age at adoption (months)	2.5 (1.7)	8.0 (8.7)	25.1 (9.6)	$F_{2,54} = 41.3$, $p < 0.0001$
Chronic stress (STICS mean score, range: 0–4)[a]	0.62 (0.34)	0.83 (0.62)	0.86 (0.60)	$F_{2,51} = 0.79$, $p = 0.46$
Perceived stress reactivity (PSRS mean score, range 0–2)[a]	0.79 (0.13)	0.82 (0.21)	0.91 (0.18)	$F_{2,51} = 2.03$, $p = 0.14$
Emotional problems[b]				Fisher's exact, $p = 0.060$
0	7 (58%)	10 (56%)	8 (32%)	
1	5 (42%)	6 (33%)	5 (20%)	
2	0	1 (5.5%)	7 (28%)	
3	0	1 (5.5%)	5 (20%)	
IQ (IQ scale)[a]	113.3 (14.5)	96.4 (18.4)	97.1 (13.3)	$F_{2,52} = 5.72$, $p = 0.006$
BMI (kg/m^2)[a]	23.7 (1.9)	24.4 (3.8)	23.1 (4.3)	$F_{2,51} = 0.64$, $p = 0.53$
High SES at age 15 (n)[c]	12 (92%)	16 (89%)	23 (92%)	Fisher's exact, $p = 1.0$
Subnourished at entry to UK (n)	–	8 (42%)	18 (72%)	Fisher's exact, $p = 0.066$

Note. Sample sizes vary for different outcomes.
[a] Mean (SD).
[b] Symptom domains endorsed by self-report.
[c] Parental socioeconomic status: skilled, managerial, technical, and professional occupations.

young adulthood.

The Short Trier Inventory for Chronic Stress (STICS; Schlotz and Schulz, 2008; Schulz and Schlotz, 1999) and the Perceived Stress Reactivity Scale (Schlotz et al., 2011) were used to assess chronic stress and stress reactivity, respectively.

2.4. Deprivation-specific problems

Deprivation-specific problems (DSP) were defined as previously reported (Kumsta et al., 2010). In brief, DSPs constitute distinctive early-appearing and persisting neuro-developmental problems associated with institutional deprivation. Individuals are categorized with DSP in the presence of either quasi-autism or disinhibited social engagement, often accompanied by ADHD and cognitive impairment.

2.5. Compliance and sampling accuracy

750 samples were returned (M = 13.2, Md = 14 per person, min = 7, max = 14, SD = 1.5). Out of those, 703 saliva samples were suitable for analysis (88% compliance rate). Participants reported a sampling time within ± 5 min of the scheduled time for 141 of 200 S2 and S3 samples (71%). For 319 out of 394 S4–S7 samples (81%), participants reported a sampling time within ± 30 min.

2.6. Cortisol storage and analysis

Participants were instructed to store used Salivettes in a refrigerator. Saliva samples were sent via pre-paid mail to the University of Southampton and stored at −20 °C. Cortisol concentrations were determined in duplicates at the Department of Genetic Psychology (Ruhr-University Bochum) using an enzyme-linked immunosorbent assay (ELISA) kit (Demeditec) according to the manufacturer's manual. Intra- and interassay coefficients of variation were below 10%.

2.7. Statistical analysis

Time trends and group differences in cortisol were modelled in one integrative model for all samples (S1–S7), and in one separate model for the CAR (S1–S3). Models were designed to estimate average effects across the two sampling days to reduce the influence of day-to-day variability (Hellhammer et al., 2007). Distributions of cortisol concentrations were positively skewed at most sampling occasions and were transformed using Box-Cox power transformations (Miller and Plessow, 2012).

2.8. Integrative model

We fitted a three-level (observations within days within subjects) functional mixed-effects regression model with linear splines (Sanchez et al., 2012) using 2 knots with $k_1 = 0.75$ h and $k_2 = 1.25$ h after awakening. The model included a full factorial design of splines by group (UK, Rom < 6, Rom > 6) and was adjusted for sex. Random intercepts at both day and subject level, and random slopes for splines 2 and 3 at day level, and spline 3 at subject level were included (random slope estimates for other splines were virtually zero). We calculated predictive margins at specific points in time that were of interest to investigate differences in cortisol levels between groups.

2.9. CAR models

We fitted a fixed-occasion mixed-effects regression model with a full-factorial design of sample (S1–S3) by group (UK, Rom < 6, Rom > 6), adjusted for sex, including a separate unstructured residual variance-covariance matrix for each day. We also computed the cortisol mean increase (MnInc = Mean (S2, S3) – S1) after awakening from untransformed cortisol values as a summary indicator of the CAR. One influential outlying observation (MnInc = 50.6) was dropped from the analysis before model estimation (criterion: Obs > Mean (MnInc) + 3 SD). The final sample comprised 80 MnInc observations from 46 individuals. We fitted a mixed-effects regression model with MnInc as outcome variable, group (UK, Rom < 6, Rom > 6) as predictor, and a random intercept on the subject level to account for non-independence of observations within subjects. To test if alterations in cortisol secretion might be a consequence of early appearing DSPs, additional models were run – given virtual absence in the other groups – within the Rom > 6 group comparing individuals with and without presence of DSP.

2.10. Models including emotional problems

To test if any group difference in cortisol secretion mediated associations between deprivation and symptoms of depression or anxiety, ordinary least squares regression models were run for CBRS Depression and Anxiety scale scores measured in young adulthood (ages 22–25

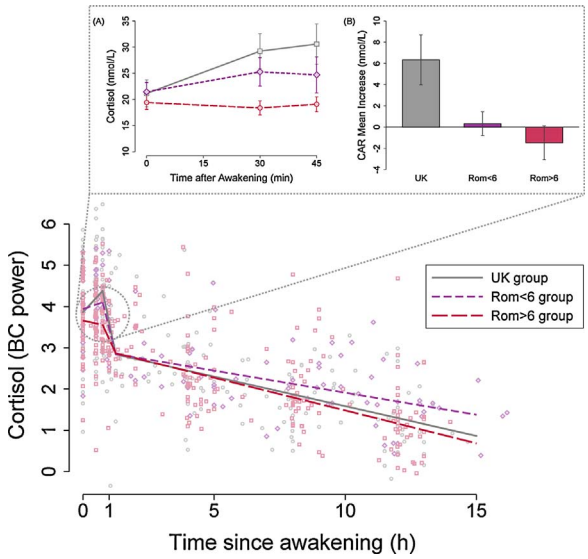

Fig. 1. Observations and trajectories for cortisol measures by time since awakening for UK adoptees and for Romanian adoptees with more (> 6 m) or less than (< 6 m) than six months of deprivation experience. The scatter plot shows sampling time and Box-Cox power transformed (BC power) cortisol measures of individual observations. Superimposed lines were plotted from group-specific fixed-effects parameters of a functional mixed-effects regression model with linear splines. Inlets (A) and (B) illustrate the cortisol awakening response (CAR) on the original scale (nmol/L). (A) Group differences in mean observed cortisol measures at awakening, and 30 and 45 min later. (B) Average mean increase (MnInc) scores for the three groups. See text for details.

years), as well as for change sores of self- and parent-reported emotional symptoms between age 15 years and young adulthood. An additional test used a grouping variable that split the Rom > 6 into those with and without presence of DSP, to test whether late-appearing problems might be a consequence of prior DSP. For any statistically significant difference in emotional symptoms between groups ($p < 0.05$) we ran path-analysis to test for potential indirect effects of deprivation on emotional symptoms via CAR MnInc, with coefficient *c* representing the initial association, coefficient *c'* the residual association, coefficient *a* the association between deprivation group and CAR MnInc, and coefficient *b* that between MnInc and the emotional symptom measure.

All models were adjusted for sex. Hypothesis tests for fixed effects were adjusted for small samples using the Kenward-Roger method. All analyses were performed using Stata v14.2. See Supplemental information 4 for details of statistical modelling and sensitivity analyses.

3. Results

3.1. Cortisol awakening response (CAR)

Fig. 1A shows average cortisol levels after awakening and Fig. 1B shows the cortisol mean increase by group on the original measurement scale (nmol/L). There was a significant main effect of time, typical for measures after awakening ($F_{2,54.2} = 19.7$, $p < 0.0001$), but no main effect of group ($F_{2,54.4} = 1.2$, $p = 0.30$). Model estimation revealed a significant sample by group interaction ($F_{4,62.9} = 3.0$, $p = 0.024$), indicating group differences in the cortisol response patterns to awakening. As shown in Fig. 1A, the UK group displayed a typical CAR, whereas in the group of Romanian adoptees with less than 6 months of deprivation experience (Rom < 6) the CAR was attenuated. In obvious contrast, the typical CAR was absent in the group of Romanian adoptees with extended deprivation experience (Rom > 6). Sample-specific contrasts revealed that average cortisol levels 30 min after awakening (S2) were significantly lower in the Rom > 6 group compared to the UK group ($F_{1,74.1} = 5.3$, $p = 0.025$), while no other two assessments differed significantly.

The CAR trajectories described above were also reflected in average MnInc scores (Fig. 1B). While the UK group showed a clear increase, average MnInc in the Rom < 6 group was close to zero, and negative in the Rom > 6 group. Model estimation revealed weak evidence for a main effect of group ($F_{2,38.7} = 2.2$, $p = 0.13$), while contrasts found that the UK group MnInc was marginally higher than that of the Rom < 6 group ($F_{1,37.3} = 3.3$, $p = 0.076$), and significantly higher than that of the Rom > 6 group ($F_{1,37.8} = 4.2$, $p = 0.049$). There were no significant differences between those with DSP and those without DSP in the CAR or in the MnInc scores (CAR sample by group interaction: $F_{2,49.7} = 0.5$, $p = 0.62$; MnInc: $F_{1,39.5} = 0.1$, $p = 0.76$). Descriptively, those with DPSs had slightly lower cortisol levels after awakening (S1: M = 19.2 nmol/L, SD = 7.0, S2: M = 17.4 nmol/L, SD = 6.9, S3: M = 17.5 nmol/L, SD = 3.6) than those without DSPs (S1: M = 19.7 nmol/L, SD = 6.3, S2: M = 19.3 nmol/L, SD = 7.4, S3: M = 19.0 nmol/L, SD = 7.2). Similar differences were observed in their mean MnInc (Rom > 6 m with DSPs: M = −1.64 nmol/L, SD = 8.35; Rom > 6 m without DSPs: M = −1.32 nmol/L, SD = 5.67).

3.2. Diurnal cortisol trajectories

Fig. 1 shows diurnal cortisol trajectories for the groups of UK and Romanian adoptees derived from the integrative model. There were no group differences in cortisol levels at awakening ($F_{2,115.8} = 0.7$, $p = 0.52$), however, model estimation revealed evidence for group differences in the trend up to 45 min after awakening ($F_{2,470.0} = 2.9$, $p = 0.054$), and in the decrease in cortisol levels between 45 min and 1 h 15 min after awakening ($F_{2,374.8} = 5.1$, $p = 0.006$). Group-specific comparisons of slopes up to 1 h and 15 min after awakening revealed significant differences between UK adoptees and the late adopted group (Rom > 6), with a less pronounced increase up to 45 min after awakening ($F_{1,462.6} = 5.9$, $p = 0.016$) and a less pronounced subsequent decrease until 1 h 15 min after awakening ($F_{1,358.7} = 9.7$, $p = 0.002$), in the Rom > 6 group. Cortisol trajectories of Romanian adoptees with limited deprivation experience (Rom < 6) did not differ from UK adoptees in either component ($F_{1,477.1}$, = 1.3, $p = 0.26$, and $F_{1,392.6} = 0.7$, $p = 0.41$). Contrasts between the Romanian adoptees groups yielded no significant difference for both increase ($F_{1,473.1} = 0.8$, $p = 0.36$) and decrease components ($F_{1,381.5} = 3.0$, $p = 0.083$).

The diurnal cortisol decline over the rest of the day was not significantly different between groups ($F_{2,90.8} = 1.2$, p = 0.30). Accordingly, testing of predictive margins showed that predicted cortisol levels 15 h after awakening were not significantly different between adoptee groups ($\chi^2(2) = 2.9$, $p = 0.24$).

3.3. Cortisol secretion and emotional problems

Regression analyses showed that self-reported emotional problem change scores between age 15 years and young adulthood were significantly larger in the Rom > 6 group than in the UK group (mean difference = 0.96, SE = 0.46, $p = 0.045$). None of the other scale scores showed significant differences (see Supplemental Fig. 1 and Supplemental Table 2). A path analysis in those $n = 28$ individuals from these groups that provided an emotional symptom change score and a MnInc measure revealed a reduction of the initial direct effect of deprivation (difference between UK and Rom > 6 groups) from $c = 0.46$ (SE = 0.21, $p = 0.028$) to $c' = 0.44$ (SE = 0.24, $p = 0.063$), a minor reduction of 4%. Whereas there was a significant association between deprivation group and CAR MnInc in the path model ($a = -5.17$, SE = 1.90, $p = 0.005$), CAR MnInc was unrelated to emotional problems change scores ($b = -0.004$, SE = 0.02, $p = 0.86$). Consequently, the indirect effect of deprivation on emotional problem change scores via CAR MnInc was not significant ($a*b = 0.02$, SE = 0.11, $p = 0.86$). In conclusion, we observed no evidence to

support a mediation model in the sub-sample investigated here.

There were no differences in the emotional problems change score between those with and without DSPs. (parent report: diff = 0.4, SE = 0.5, $p = 0.44$; self-report: diff = 0.6, SE = 0.4, $p = 0.15$). However, scores on both Conner's Depression and Anxiety scales in young adulthood were significantly higher in the Rom > 6 subgroup with DSP (Depression: diff = 1.2, SE = 0.5, $p = 0.024$; Anxiety: diff = 1.1, SE = 0.5, $p = 0.031$). Since DSP did not show an association with the CAR, we did not run mediation models for these differences.

4. Discussion

There is growing evidence that bio-behavioural systems involved in adaptations to stress, including the HPA axis, represent targets for the effects of adverse childhood experiences. Here, we show that time-limited exposure to severe institutional deprivation in early childhood followed by adoption into nurturing families is associated with altered HPA axis regulation in young adulthood. Specifically, the typical pattern of the cortisol curve after awakening was completely absent in the group of young adults who experienced extended deprivation (between 6 and 43 months). Deprivation specific problems, defined as complex pattern of persisting neuro-developmental problems observed in about half of the late-adopted Romanians, were associated with increased depression and anxiety scores but were not related to cortisol secretion patterns. Adoptees who spent less than 6 months in the institution showed an attenuated CAR pattern compared to the non-deprived UK comparison group, who displayed the typical rise in cortisol 30 and 45 min after awakening.

The CAR is considered an important biomarker in stress research as it combines features of a reactivity index with aspects tied to circadian regulation, expressed as part of normal circadian physiology (Stalder et al., 2016). Group differences in the CAR with a complete lack of a response in the most severely deprived and an attenuated pattern in the group with short duration of deprivation are consistent with the fairly uniform observation of a pattern of hypocortisolism in institutionalized and postinstitutionalized children at much younger ages. Our findings are also consistent with a previous finding that late removal from institutions (between 18 and 24 months) is associated with hyporeactivity in response to stress, while the same is less apparent with earlier removal (Koss et al., 2016; McLaughlin et al., 2015).

Whereas we observed clear differences in cortisol patterns after awakening, there were no differences in diurnal cortisol secretion patterns between groups across the remainder of the day. It is of note, however, that group differences in cortisol secretion extended beyond the CAR, with the late adopted group showing a less pronounced decline in cortisol levels between 45 and 1 h and 15 min after awakening.

Given evidence from prior studies that alterations in HPA activity mediate between adverse rearing conditions and symptoms of psychopathology, we further asked whether differences in cortisol regulation after awakening might be related to emotional problems in adulthood. Path analyses provided inconclusive evidence with regard to the role of CAR alterations in mediating the link between institutional deprivation and emotional problems. Given that the sub-sample with available data for both cortisol and change scores in emotional problems was underpowered to detect small to medium mediation effects, it cannot be ruled out that the effects of deprivation on emotional problems might be mediated via HPA axis dysregulation. It is also possible that altered HPA axis activity might constitute a vulnerability marker increasing risk for affective disorders only in combination with further stress experience, and therefore influence mental health outcomes not until later in life. Mechanistically, relative hypocortisolism may result in diminished counter-regulatory control of the sympathetic nervous system (SNS) and the immune system, leading to hyperactivity in these systems (Fries et al., 2005).

Our results show that severe psychosocial deprivation experienced in early childhood is associated with alterations in the regulation of the HPA axis about 20 years after adoption. It cannot be ruled out that disturbed cortisol regulation is a late appearing consequence of institutional deprivation. However, given findings by other groups who have prospectively assessed HPA axis function in institutionalized and post-institutionalized children (Koss et al., 2016; McLaughlin et al., 2015), and given our own findings of early appearing deprivation-specific problems which are highly persistent and are not eradicated by the overall very positive post-adoption experience, it is likely that HPA axis dysregulation also constitutes an early appearing and stable phenotype associated with institutional rearing. In light of the evidence of decreased cortisol activity and reactivity in adults and children with experience of early adversity (Carpenter et al., 2007; Elzinga et al., 2008; Lovallo et al., 2011; Miller et al., 2007; Power et al., 2012; Schwaiger et al., 2016), it has been proposed that sustained periods of chronic stress, such as psychosocial deprivation and neglect, are initially associated with high levels of circulating cortisol, followed by counter-regulation which eventually results in decreased HPA axis re-/activity. This attenuation hypothesis (Trickett et al., 2010) is supported by a meta-analysis showing that the more time that had elapsed since trauma emerged, the lower a person's cortisol levels (Miller et al., 2007).

Epigenetic alterations have been proposed as a mechanism for the stability of the long-term effects of early environmental influences in general (Mill and Heijmans, 2013) and the long-term setting of the HPA axis in particular (Chen et al., 2012; Murgatroyd et al., 2009; Turecki and Meaney, 2016; Weaver et al., 2004; Zhang and Meaney, 2010). Our own pilot study showed differences in DNA methylation patterns between the late adopted and early adopted groups, with large DNA methylation differences across several CpG sites in the promoter region of the cytochrome P450 2E1 gene (*CYP2E1*), involved in cholesterol and steroid synthesis, amongst other functions (Kumsta et al., 2016). However, our epigenome-wide scan did not provide evidence for differences in DNA methylation of HPA axis related genes. Generally, it has to be kept in mind that epigenetic processes are by definition highly tissue specific, and that animal studies found DNA methylation differences in brain regions involved in HPA axis regulation. It is thus possible that differences in DNA methylation do mediate the effects of early adversity on stress regulation, but that DNA methylation differences are not reflected in accessible peripheral tissue (Mill and Heijmans, 2013).

The following limitations need mention. Power was limited due to relatively small sample sizes, several missing time records, and only two days of assessment. The compliance rate of 88% was acceptable, whereas sampling accuracy overall was at a moderate level (S2 and S3: 71%; S4–S7: 81%), despite face-to-face instructions and intensive measures to support accurate and reliable sampling. Furthermore, we did not use electronic monitoring devices to verify time of awakening or times of sample collection. Similar levels of cortisol at S1, however, indicate that a systematic delay between awakening and initiation of sampling is unlikely. We dropped all S1 observations from our CAR analysis that had no recorded time of awakening, and we dropped all post-awakening observations (S2 and S3) that had no recorded time or had a recorded time that deviated more than 5 min from the sampling design. Despite the lack of objective verification we are confident that those samples used were taken at the time intended by our sampling design. As our assessments of emotional problem at age 15 and in young adulthood used slightly different item wording, and we have not tested for measurement invariance, our emotional problems change score might in part reflect differential responses to item wording in addition to true change in emotional problems.

It should further be noted that the 6-months cut-off used here is likely going to be specific for ERA study. It cannot be assumed that the same would apply to the effects of less severe deprivation, institutional care more generally, or other types of abuse or neglect in non-institutional samples. For instance, in participants of the Bucharest Intervention Project who grew up in the same institutions as the ERA

children but during the later post-Communist era and where hence exposed to less severe deprivation, lasting effects on HPA axis regulation were found only in those adoptees who were removed from institutions later than 2 years.

5. Conclusions

In conclusion, we report results of a most unusual "natural experiment", which prospectively followed the development of children adopted out of severely depriving Romanian institutions. The design allowed investigating the long-term effects of timing and length of deprivation on outcomes, and we show that extended psychosocial deprivation in early childhood is associated with a distinct lack of the cortisol awakening response, whereas shorter duration of deprivation is associated with an attenuated CAR. These results support the notion that adverse rearing circumstances can have long-term effects on HPA axis regulation.

Conflict of interests

Edmund Sonuga-Barke reports having received speaker fees, consultancy, research funding, and conference support from Shire Pharma and speaker fees from Janssen Cilag. He has received consultancy fees from Neurotech Solutions, Aarhus University, Copenhagen University and Berhanderling, Skolerne, Copenhagen, Katholieke Universiteit Leuven, and book royalties from Oxford University Press and Jessica Kingsley. His university receives financial support and he receives an honorarium from Wiley for editorship. Robert Kumsta, Wolff Schlotz, Dennis Golm, Dirk Moser, Mark Kennedy, Nicky Knights, Jana Kreppner, Barbara Maughan and Michael Rutter reported no biomedical financial interests or potential conflicts of interest.

Acknowledgements

We thank our advisory board for all their useful suggestions, especially Megan Gunnar (University of Minnesota, MN, USA). We would like to express our sincere gratitude to all the families and young people who have participated in this study over the many years it has been running. Without them this work would not be possible. This work was supported by a project grant from the UK Economic and Social Research Council (ESRC; RES-062-23-3300).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.09.021.

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